Fall 1992 Second Mid Term Chem130A Wemmer

1. A protein was eluted from a chromatography column with a 1M NaCl solution. The protein concentration in this fraction is roughly 10mM. You are trying to reduce the salt content of your protein solution by bag dialysis against 0.1M NaCl. Assume that the dialysis membrane is permeable for salt ions and water, but not for protein.

a.Estimate the initial osmotic pressure at 25°C. State your assumptions.

b. As you do the experiment, you observe that the volume of your protein solution in the bag increases rapidly. You are afraid that the bag might burst, although you made your bag large enough to easily hold 150mL. Assuming that the dialysis membrane is only permeable to water but not to salt, what will be the maximum volume of your protein solution at equilibrium? Initially you started with 10mL of solution. You can assume that the NaCl concentration on the outside does not change during the experiment. Ignore any pressure that the bag might exert on the solution. State your assumptions.

4. For a protein called T4 lysozyme the gene sequence was modified so that two large, hydrophobic amino acids were replaced with the much smaller residue alanine. It was observed that a hole was left in the hydrophobic interior of the protein. When the researchers added benzene to the solution, they found that one benzene molecule did bind in the hole, and made the protein more stable. They know that benzene is slightly soluble in water (2.2×10^{-2} moles/Liter in a saturated solution at 25°C).

a. What is the ΔG° for converting one mole of pure benzene to one mole of benzene dissolved in water?

b. Make an estimate for the free energy of binding of benzene to the protein based on these data. Explain your reasoning.

c. It was found that the dissociation constant for the benzene from the protein was 4×10^{-4} M. Does this agree with your estimate?